Abstract

There is increasing interest in using live cell imaging to monitor not just individual intracellular endpoints, but to investigate the interplay between multiple molecular events as they unfold in real time within the cell. A major impediment to simultaneous acquisition of fluorescent signals from multiple probes is that emission spectra of many fluorophores overlap, often with maxima that are only a few nanometers apart. **Spectral** acquisition of mixed fluorescence signals captured within a dedicated scanning range can be used to quantitatively separate signals into component spectra. We report here the development of a novel live cell application of **spectral unmixing** for the simultaneous monitoring of intracellular events reported by closely-emitting fluorophores responding dynamically to external stimuli. We validate the performance of dynamic **spectral unmixing** microscopy (DynSUM) using genetically encoded sensors to simultaneously monitor changes in glutathione redox potential (Egsh) and H_2O_2 production in living cells exposed to oxidizing and reducing agents. We further demonstrate the utility of the DynSUM approach to observe the relationship between the increases in Egsh and H_2O_2 generation induced in airway epithelial cells exposed to an environmental electrophile.